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The influence of allotypes on the IgG subclass response to chromosomal β-lactamase of *Pseudomonas aeruginosa* in cystic fibrosis patients

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SUMMARY

Sera from 70 adult cystic fibrosis (CF) patients with chronic lung infection with *Pseudomonas aeruginosa* were typed for seven GM and two KM allotype determinants. IgG class and all four IgG subclasses of antibodies against chromosomal β-lactamase of *Ps. aeruginosa* (aβab) were measured in all 70 CF patients in a cross-sectional study. The aβab IgG subclass response in sera collected during the first 11 years of chronic infection from 20 CF patients (10 patients with *G3M*5 G1M*3/G3M*5 G1M*3* genotype and 10 patients with *G3M*21 G1M*1/G3M*21 G1M*1* genotype) was analysed in a longitudinal study. Increased levels of IgG2 were associated with the presence of GM 23 allotype. IgG3 aβab levels were the lowest for subjects with the GM 1,2,3,17 23 5,21 and GM 1,3,17 21 phenotypes and the highest in subjects with GM 3,23,5 and GM 3,5. No significant differences in IgG1 and IgG4 aβab levels were found between the different phenotypes. IgG1 aβab levels were higher in patients with *KM*3/KM*3* genotype compared with patients with *KM*3, *1* genotype. Patients with *G3M*5 G1M*3/G3M*5 G1M*3* genotype had in both the cross-sectional and the longitudinal study higher IgG3 aβab, lower IgG4 aβab levels and poorer lung function than patients with *G3M*21 G1M*1/ G3M*21 G1M*1* genotype. An influence of the allotypes on the clinical course of chronic lung infection with *Ps. aeruginosa* in patients with CF is suggested.

Keywords GM allotype IgG subclasses antibodies against β-lactamase *Pseudomonas aeruginosa* cystic fibrosis

INTRODUCTION

Chronic infection of the lower respiratory tract with *Pseudomonas aeruginosa* is the main cause of morbidity and mortality among patients with cystic fibrosis (CF) [1]. Intensive anti-pseudomonal treatment has significantly improved the survival of CF patients [2].

The repeated use of β-lactam antibiotics led to the emergence of resistant *Ps. aeruginosa* strains, making the treatment less efficient [3–5]. Production of high levels of chromosomal β-lactamase is the main resistance mechanism [6].

We have previously shown that CF patients chronically infected with resistant *Ps. aeruginosa* strains produce antibodies against the chromosomal β-lactamase of *Ps. aeruginosa* (aβab) of all IgG sublcasses [7,8]. In previous studies we found that high IgG antibody levels against *Ps. aeruginosa* antigens (especially IgG2 and IgG3 antibodies) correlated to poor prognosis, probably due to a more efficient activation of complement and thereby of the inflammatory cascade [9–11]. In contrast, our previous results showed that patients with high aβab levels in the early stages of infection developed good lung function [8]. This raises the possibility that the IgG allotypes of the patients, which are known to correlate to the IgG subclasses, may also play a role in the IgG subclass response to β-lactamase, and possibly in the lung function of the patients.

The present study was therefore planned to analyse the influence of allotypes on the IgG isotypes of aβab and the lung function of patients with CF.

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MATERIALS AND METHODS

Immunoglobulin allotypes

The genetic markers of GM allotypes are expressed as antigens on the constant region of the heavy chains of IgG1, IgG2 and IgG3 immunoglobulins (chromosome 14) and KM allotypes on κ light chains (chromosome 2). They are inherited as a group, called haplotype, in a codominant Mendelian way. While GM 23 is the only allotypic marker for IgG2, in Caucasians the *G1M*1* and *G1M*3* act as alternative allele for IgG1 and *G3M*5* and *G3M*21* for IgG3. KM*3 and KM*1 are alternative alleles for κ light chains.

Immunoglobulin allotypes were determined by the haemagglutination-inhibition method [12]. Samples were typed for G1M 1,2,3,17, G2M 23, G3M 5,21 and KM1,3. The notation follows the international system for human gene nomenclature [13].

Briefly, the method employs human blood group Orh+ erythrocytes coated with anti-Rh antibodies of known GM allotype and a panel of monospecific anti-allotype sera. Test sera containing immunoglobulin of particular allotype inhibit haemagglutination of anti-allotype antibody, whereas negative sera do not.

Determination of IgG antibodies in serum

Total IgG subclasses. Total levels of serum IgG1,2,3 and 4 were measured by single radial immunodiffusion using polyclonal rabbit antisera, as previously described [14].

Aβab-specific IgG. These were measured by a previously published ELISA method [7].

Aβab-specific IgG subclasses.

1. *ELISA for aβab-specific IgG subclasses.* IgG subclass aβab response was measured by four ELISAs. Ninety-six-well microtitre plates (Maxisorb; Nunc, Roskilde, Denmark) were coated with purified β-lactamase (2 μg/ml) diluted in 0·1 M sodium carbonate buffer pH 9·6 by overnight incubation at 4°C. Additional binding sites were blocked by incubation for 2 h at 37°C with 5% fetal calf serum (FCS) diluted in the carbonate buffer. Serum samples were diluted 1:100 and 1:1000 in dilution buffer (Na-phosphate buffer+ 1% Tween 20). Samples were allowed to react overnight. Horseradish peroxidase-labelled anti-IgG subclass-specific MoAbs (IgG1, HP 6069; IgG2, HP 6014; and IgG4, HP 6025; CLB, Amsterdam, The Netherlands) were added to each well and incubated for 2 h. For IgG3 level determinations the unlabelled monoclonal anti-IgG3 (HP 6050; CLB) was used and a peroxidase conjugate rabbit anti-mouse antibody, diluted 1:1000 (P260; Dako, Glostrup, Denmark) was used as secondary antibody in the ELISA. MoAbs were diluted 1:5000 for IgG1, 1:500 for IgG2, 1:1000 for IgG3 and 1:2000 for IgG4. Each assay was calibrated after a standard curve constructed from eight double dilutions of a standard serum. In order to obtain a value of 1·5 for the optical density (OD) of the lowest dilution of the standard serum, the dilutions started at 1:100 for the IgG1, IgG3 and IgG4 and 1:40 for the IgG2 ELISA.

Sodium citrate chloride (0·1 M, pH 5) containing 1,2-phenylenediamine (2·2 mM) (Dako) and H_2O_2 (6·5 mM) was added per well, and enzyme reaction was stopped by addition of H_2SO_4 (1 M) after incubation in the dark for 30 min. The OD at 492 nm was read on an automatic plate reader.

All serum samples were tested in duplicate. If the test sample had an OD value exceeding the upper limit of the reader, the sample was diluted 1:2000 and 1:4000 and retested. The standard serum was a pool of 10 chronic infected patients with CF, with high aβab titres, as measured in ELISA. Results were expressed in ELISA units (EU) and calculated from the standard curve using multiple regression analysis and corrected by the dilution factor.

2. *Specificity of the MoAbs to human IgG subclasses.* The mouse MoAbs to human IgG1–4 were of World Health Organization reference quality and displayed the desired specificity in immunoassays [15].

3. *Reproducibility of the assay.* Ten serum samples from CF patients with chronic *Ps. aeruginosa* infection with low, medium and high aβab levels were tested in duplicate in two different plates on two different days. The intraplate, plate-to-plate and day-to-day variations were determined using the formula $\text{s.d.} = \sqrt{(\sum d^2/2n)}$, where s.d. is the standard deviation, $\sum d^2$ is the sum of squared differences of double determinations of the same sample, and n is the number of observations. The intraplate variation for IgG1, 2, 3, 4 was 2·2%, 7·2%, 0·3%, 3·6%, plate-to-plate variation 4·1%, 8·6%, 0·5%, 7·6% and day-to-day variation 9%, 9·7%, 3·5% and 9·4%.

CF patients. Diagnosis of CF was established on the basis of abnormal sweat electrolytes, characteristic clinical features and CF genotype. Patients were seen monthly in the out-patient clinic for evaluation; pulmonary function tests and bacteriological investigations were carried out.

The infection was defined as chronic when cultures of monthly sputum samples yielded *Ps. aeruginosa* for 6 months consecutively and/or two or more precipitins against *Ps. aeruginosa* were present in the serum [16].

A group of 70 adult CF patients from the Danish Centre were included in a cross-sectional study and 20 CF patients in a longitudinal study.

1. *Cross sectional study.* In order to avoid differences in management of chronic *Ps. aeruginosa* lung infection in different periods of time, we chose a group of CF patients (mean age 26 ± 6 years) with duration of chronic infection between 8 and 17 years. This group of patients was admitted every 3 months for 2-week courses of i.v. anti-pseudomonal treatment once the chronic infection was established. The anti-pseudomonal treatment consisted of a combination therapy of an aminoglycoside with a β-lactam antibiotic. This treatment regimen has been used in our centre since 1976 [17].

Sputum samples obtained by expectoration or endolaryngeal suction were Gram-stained and examined under the microscope to confirm their origin from the lower airways based on the absence of squamous epithelial cells and the presence of ciliated epithelial cells plus polymorphonuclear leucocytes. The material was cultured as previously reported [18]. Pulmonary function (forced vital capacity (FVC) and forced expiratory volume in the first second (FEV_1)) were determined in patients ≥6 years of age at each monthly visit using an electronic spirometer (Spirotron, Dräger, Denmark). All values were expressed as percentage of the expected values according to height and sex. Individual values in this study were those determined at the time of serum sampling. Lung function was considered good when FEV_1 values ≥70% and poor when FEV_1 <70% of the expected values for height and sex. Data from patients have been recorded prospectively using the flow sheets described previously [19]. Data on lung function were not available for two of the 70 CF patients.

The patient population was divided into groups, according to the most probable genotypes: (i) homozygotes for both G1M and G3M markers *G3M*5 G1M*3/G3M*5 G1M*3* or *G3M*21 G1M*1/G3M*21 G1M*1*; (ii) heterozygotes for both G1M and G3M markers *G3M *5, *21 G1M *1, *2, *3, *17*; (iii) homozygotes for G3M, *G3M*5/G3M*5* or *G3M*21/G3M*21* and heterozygotes for G1M *G1M *1, *2, *3, *17*; (iv) homozygotes for G1M *G1M*3/G1M*3* or *G1M*1, *17/G1M*1, *17* and heterozygotes for G3M *G3M*5, *21*.

2. *Longitudinal study.* Sera collected every year during 11 years of chronic infection with *Ps. aeruginosa* from 20 patients homozygotes for both G1M and G3M markers were longitudinally analysed for IgG aβab subclass development. Ten CF patients were *G3M*5 G1M*3/G3M*5 G1M*3* homozygotes and 10 were *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes. The mean age of the patients at the start of chronic infection was 9 ± 3 years for

Table 1. The distribution of IgG aβab subclass and GM phenotypes in 70 chronic infected cystic fibrosis patients (results expressed in the respective ELISA units as median of groups)

		Anti-β-lactamase antibodies (ELISA units)				
GM phenotypes	Number of patients	IgG1	IgG2	IgG3	IgG4	IgG
1,2,3,17 23 5,21	6	57 185	167 767*	0	3485	58·5
1,2,3,17 5,21	4	95 415	4522*	3·3	3183	17·2
1,3,17 23 5,21	13	65 932	11 556†	11·8	7592	45
1,3,17 5,21	5	65 646	2258†	1	7966	32
1,3,17 21	3	92 282	2064	0	72 089	35
3 23 5	16	69 663	16 850	29·3	2132	40
3 5	8	61 802	16 336	27·1	2829	50·5
1,17 21	3	116 099	0	12·5	8915	27
1,2,17 21	8	57 565	14 023	2·5	6369	44·5
1,17 23 5,21	1	11 588	191 662	14	42 704	337
1,17 23 21	1	82 857	353	84	29 327	19
1,2,3,17 23 5	1	57 566	10 123	180	1373	53
1,3,17 23 21	1	601 676	0	0		71

* $P = 0{\cdot}002$.
† $P = 0{\cdot}03$.

*G3M*5 G1M*3/G3M*5 G1M*3* homozygotes and 8 ± 4 years for *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes. All patients received similar anti-pseudomonal treatment, and the *Ps. aeruginosa* strains developed resistance to β-lactam antibiotics. Pulmonary function was determined at each monthly visit, and values used in this study are the mean of all results within the observation period. For practical purposes the duration of chronic infection was expressed in seven observation periods: 0–1, 2–3, 3–4, 4–5, 5–7, 7–9 and 9–11 years from the onset of chronic infection. Patients with poor (good) lung function were considered those with $FEV_1 \leq 70\%$ ($FEV_1 > 70\%$) in at least six of the eight observation periods of the chronic infection.

Statistical analysis. Single- or two-factor analysis of variance (ANOVA) and Kruskal–Wallis test for non-parametric data were used when more than two groups were compared. The Mann–Whitney test was used for non-parametric unpaired data where two groups were compared. χ^2 test was used for comparison of prevalences. Calculations were performed on an Apple Macintosh, using Stat View TM 512 as software. For the ANOVA tests data were log transformed before calculations. Their level of significance was 5% for two-tailed comparison.

RESULTS

Cross-sectional study

Phenotypes. The distribution of the GM phenotypes observed in the population of 70 CF patients was published before, and it was comparable to healthy populations [20].

The median levels of aβab (IgG and IgG subclasses) in 70 CF patients with various phenotypes after a mean of 15 (8–20) years from the onset of chronic *Ps. aeruginosa* infection are shown in

Table 2. IgG aβab subclass in 70 patients according to their genotypes (results expressed in the respective ELISA units as median of the groups)

Genotypes	Number of patients	IgG1	IgG2	IgG3	IgG4
*G3M*5 G1M*3* G3M*5 G1M*3	24	65 732	16 336	26·6†‡	2672
*G3M*21 G1M*1* G3M*21 G1M*1	12	88 101	10 158	8·65†	8218
G1M*1, *2, *3, *17 G3M*5, *21	34	65 932	8680	0‡	4320
GM 23	39	65 932	15 741	18·2	4552
Lack of GM23	31	82 655	9767	8·3	4734
*KM*3* KM*3	58	77 005*	10 408	14·8	6401
KM *1, *3	12	34 412*	16 850	8	2066

* $P = 0{\cdot}04$.
† $P = 0{\cdot}03$.
‡ $P = 0{\cdot}0002$.

Table 3. Total IgG subclass serum concentration in 70 patients according to their genotypes (results expressed in mg/ml as median (ranges) of the groups)

Genotype	Number of patients	Total serum concentration (mg/ml)			
		IgG1	IgG2	IgG3	IgG4
*G3M*5 G1M*1* / G3M*5 G1M*1	24	7·7 (5·2–20)	4·1 (1·7–8·1)	1* (0·3–2·5)	1·7 (0·1–5·4)
*G3M*21 G1M*1* / G3M*21 G1M*1	12	8·1 (5·2–13·5)	2·7 (1·7–6·3)	0·4* (0·2–0·8)	1·5 (0·3–3·9)
G1M*1, *2, *3, *17 G3M*5, *21	34	8 (4·9–21·2)	3 (1·3–8·2)	0·7 (0·1–3·6)	1·5 (0·001–4)
GM 23	39	8 (4·9–20)	3·1 (1·3–8·2)	0·8† (0·1–3·6)	1·6 (0·1–5·4)
Lack of GM23	31	8 (5·2–21·2)	3·7 (1·7–8·1)	0·5† (0·2–1·5)	1·7 (0·01–3·9)
*KM*3* / KM*3	58	8·1 (4·9–21·2)	3·7 (1·3–7·1)	0·7 (0·1–2·5)	1·7 (0·01–5·4)
KM*1, *3	12	8 (5·2–18·3)	2·8 (1·7–8·2)	0·5 (0·3–3·6)	0·85 (0·6–3·2)

* $P = 0{\cdot}0005$.
† $P = 0{\cdot}002$.

Table 1. The patient population consisted of 13 different phenotypes. The four phenotypes including only one patient have been excluded from the calculations.

There was no statistically significant difference either in the IgG aβab levels or in IgG1 or IgG4 aβab levels between the different phenotypes.

1. *IgG2 aβab.* Significantly higher IgG2 levels were found in patients positive for G2M 23 than in those who were negative for this marker. Thus, there was a significant difference ($P = 0{\cdot}002$) in IgG2 aβab levels between the phenotypes GM 1,2,3,17 23 5,21 and GM 1,2,3,17 5,21 and between the phenotypes GM 1,3,17 23 5,21 and GM 1,3,17 5,21 ($P = 0{\cdot}03$). However, no significant difference in IgG2 levels was found between phenotypes GM 3 23 5 and GM 3 5.

2. *IgG3 aβab.* IgG3 aβab levels were lowest for subjects with the GM 1,2,3,17 23 5, 21 and GM 1,3,17 21 phenotypes, and highest in subjects with GM 3, 23,5 and GM 3,5.

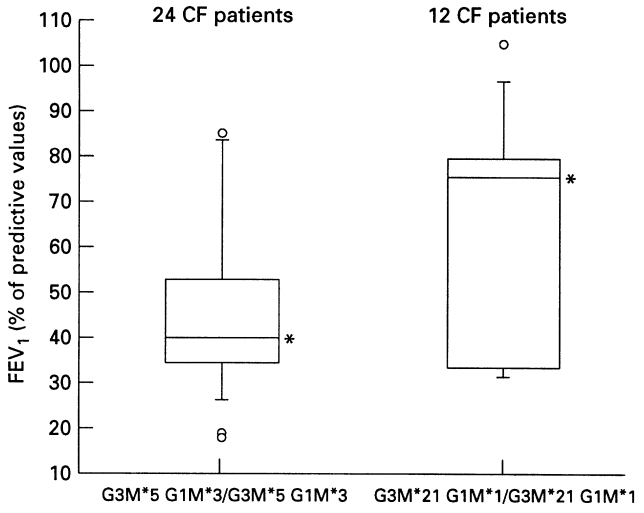

Fig. 1. Box and whisker plot representing the median, 25, 75 and 90 centiles of the lung function (FEV_1 as percent of the predictive values) in 24 cystic fibrosis (CF) patients with *G3M*5 G1M*3/G3M*5 G1M*3* genotype and in 12 CF patients with *G3M*21 G1M*1/G3M*21 G1M*1* genotype. *$P = 0{\cdot}02$.

Genotypes. Data on median values of IgG subclass aβab levels according to the different genotypes are presented in Table 2. IgG3 aβab median levels were higher in *G3M*5 G1M*3/G3M*5 G1M*3* homozygous sera compared with heterozygotes ($P = 0{\cdot}0002$) and with the *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes ($P = 0{\cdot}03$) (Table 2). IgG4 aβab median levels were higher in *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes than in *G3M*5 G1M*3/G3M*5 G1M*3* homozygotes, though not statistically significant (Table 2).

The influence of the KM markers on subclass aβab levels was calculated, and we found that *KM*3/KM*3* homozygotes had significantly higher levels of IgG1 aβab ($P = 0{\cdot}045$) compared with *KM*3/KM*1* heterozygotes (Table 2).

When total serum IgG subclass levels were analysed, a significant difference in total IgG3 levels was found between *G3M*5 G1M*3/G3M*5 G1M*3* and *G3M*21 G1M*1/G3M*21 G1M*1* homozygous sera ($P = 0{\cdot}0005$), and between the G2M 23 positive and negative sera ($P = 0{\cdot}002$) (Table 3).

*Genotypes and lung function. G3M*21 G1M*1/G3M*21 G1M*1* homozygotes ($n = 12$) had significantly better lung function (FEV_1 values) than *G3M*5 G1M*3/G3M*5 G1M*3* homozygotes ($n = 24$) ($P = 0{\cdot}02$) (Fig. 1). Patients with poor lung function ($n = 44$) had significantly higher total serum IgG1, 2 and 3 levels than those with good lung function (Table 4).

When the specific aβab IgG subclasses were analysed, only IgG3 aβab levels were significantly higher in patients with poor lung function compared with those with good lung function ($n = 24$) (Table 5).

Longitudinal study

The development of the IgG1–4 aβab during the course of chronic lung infection in 10 *G3M*21 G1M*1/G3M*21 G1M*1* and 10 *G3M*5 G1M*3/G3M*5 G1M*3* homozygote patients is shown in Fig. 2a–d. There was no significant difference in the development of IgG1 aβab and IgG2 aβab between the two groups of patients. Patients homozygotes for *G3M*5 G1M*3/G3M*5 G1M*3* developed significantly higher IgG3 aβab levels compared with *G3M*21 G1M*1/G3M*21 G1M*1* homozygote patients ($0{\cdot}008 < P < 0{\cdot}04$). In contrast, *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes patients developed predominantly IgG4

Table 4. Total IgG subclass levels (median values and ranges) in patients with poor ($n = 24$) and good ($n = 44$) lung function (1.f.)

	IgG total (mg/ml)	
	Good l.f., $n = 24$	Poor l.f., $n = 44$
IgG1	6·9* (4·9–11·4)	9·3* (5·8–21·2)
IgG2	2·6† (1·3–4·9)	4† (1·5–8·2)
IgG3	0·4‡ (0·1–1·5)	0·8‡ (0·2–3·6)
IgG4	1·1 (1–3·9)	1·7 (0·01–5·4)

*‡ $P = 0{\cdot}004$.
† $P = 0{\cdot}02$.

aβab, though there was no statistically significant difference in IgG4 aβab levels between the two groups of patients. Patients with poor lung function were significantly ($P < 0{\cdot}05$) more prevalent in the *G3M*5 G1M*3/G3M*5 G1M*3* homozygote group (seven out of 10 patients) than in the *G3M*21 G1M*1/G3M*21 G1M*1* homozygote group (two out of 10 patients).

DISCUSSION

In spite of the intensive anti-pseudomonal treatment, chronic *Ps. aeruginosa* infection runs a highly variable course in individual patients, some of whom remain in good clinical condition with only slight progression in pulmonary damage over a period of many years, while others experience a rapid downhill course, terminating in irreversible respiratory insufficiency. The pulmonary damage is believed to be due to inflammatory reactions secondary to local immune complex formation [21–23].

High levels of specific anti-pseudomonal antibodies correlate with poor prognosis [16,24]. In contrast, the early development of high levels of aβab were associated with good lung function in some CF patients [8], suggesting that these antibodies could possibly have a beneficial role in β-lactam treatment by inhibiting β-lactamase activity.

In the present study, CF patients with *G3M*5 G1M*3/G3M*5 G1M*3* genotype had higher IgG3 aβab, lower IgG4 aβab levels and a poorer lung function than patients with *G3M*21 G1M*1/ G3M*21 G1M*1* genotype. This suggests that differences in genetic determination of the immune response could explain the variable course of the lung disease among CF patients. Our results are in agreement with previous studies showing an association between anti-pseudomonal-specific IgG3 levels and poor lung

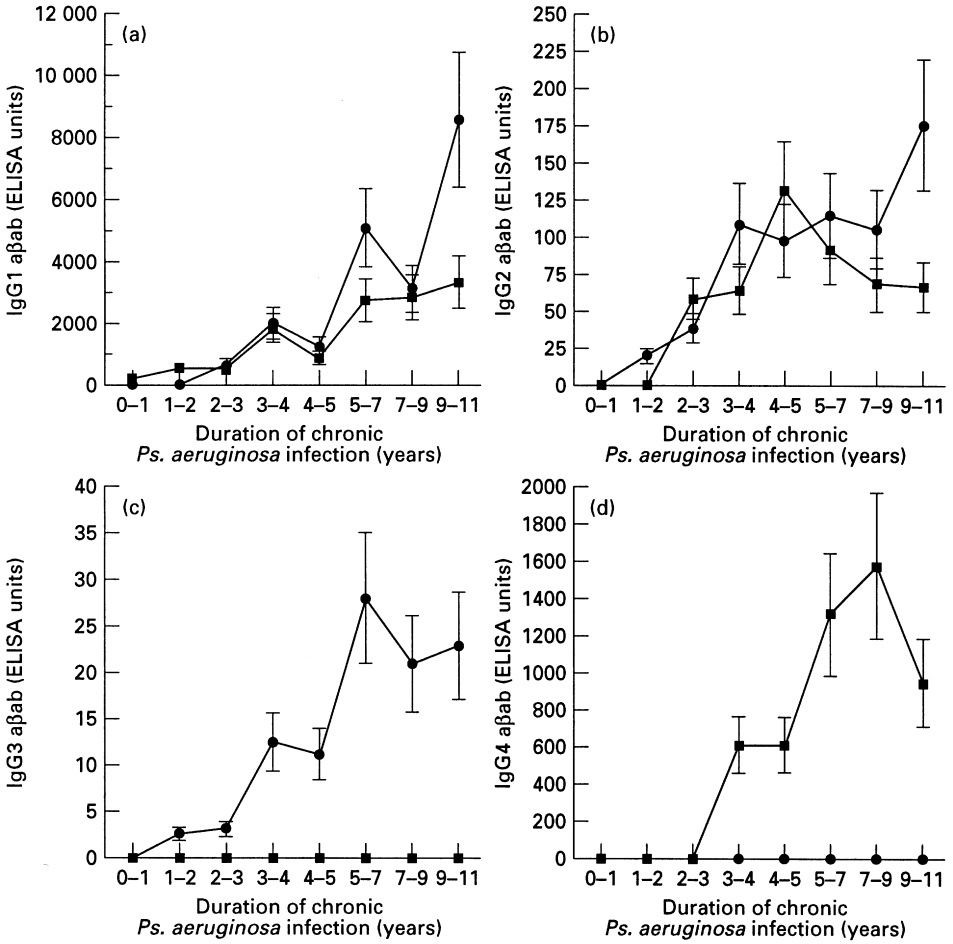

Fig. 2. Development of IgG1 (a), IgG2 (b), IgG3 (c) and IgG4 (d) aβab during 11 years of chronic *Pseudomonas aeruginosa* infection in 10 patients with *G3M*5 G1M*3/G3M*5 G1M*3* genotype (●) and 10 patients with *G3M*21 G1M*1/G3M*21 G1M*1* genotype ■. Each point represents the median values with 25% variation interval.

Table 5. Anti-β-lactamase-specific IgG subclass levels (median values and ranges) in patients with poor ($n = 24$) and good ($n = 44$) lung function (l.f.)

	IgG aβab (ELISA units)	
	Good l.f., $n = 24$	Poor l.f., $n = 44$
IgG1 aβab	66 530	70 975
IgG2 aβab	9945	13 121
IgG3 aβab	0*	19·6*
IgG4 aβab	5217	3485

* $P = 0{\cdot}006$.

function or poor prognosis [9,10,25–27]. On the other hand, the association of high IgG3 levels with the *G3M*5 G1M*3/G3M*5 G1M*3* genotype is now well established [28–30]. In our study, patients with poor lung function had significantly higher levels of total IgG1, 2 and 3 than patients with good lung function. However, only IgG3 aβab were at significantly higher levels in patients with poor lung function. This association is probably due to the complement-activating capacity of this IgG subclass [11].

In contrast to IgG3 aβab, IgG4 aβab were found in higher levels in *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes compared with *G3M*5 G1M*3/G3M*5 G1M*3* homozygotes. The same association was noted by Pressler *et al.* [20] for IgG4 anti-*Ps. aeruginosa* lipopolysaccharide (LPS) antibody levels, which were higher in *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes compared with *G3M*5 G1M*1/G3M*5 G1M*1* homozygotes.

Although until now no allotypic markers have been described for IgG4, there have been some suggestions in earlier studies [31] that the phenotype GM 3 23 5 might exert a positive effect on IgG4 serum concentration in normal sera, paralleling the presence of the GM 23 marker [29]. However, this relationship could not be demonstrated in normal sera, either by Van der Giessen *et al.* [32] and Pandey & French [33], or by our findings on specific IgG4 aβab in patients with CF. In contrast, our observations suggest that in CF patients the linkage between *G1M*1* and *G3M*21* could influence IgG4 aβab levels. Some studies [11,34,35] suggest a possible protective role of the IgG4 antibodies. This could be explained by the properties of IgG4 as a poor complement-fixing, poorly opsonizing, non-precipitating antibody. As such, it is probably less important in immune complex-mediated lung damage in patients with CF and chronic pulmonary *Ps. aeruginosa* lung infection.

As previously reported for normal sera [32,36,37], the presence of the IgG2 allotypic marker G2M 23 determined significantly higher serum IgG2 aβab levels compared with sera which were negative for this marker.

The highest IgG2 aβab levels were found in GM 1,2,3,17 23 5,21 phenotype, and this is in high accordance with Pandey & French's results [33] on IgG2 concentration in healthy adults. Some of the heterozygote phenotypes previously reported in association with lower concentrations of total IgG3 in normal adults [33] or with low IgG3 antibody levels against protein antigens [20,38] were also found in our study to associate with low IgG3 aβab. This suggests that besides the *G3M*21 G1M*1/G3M*21 G1M*1* homozygotes, known as low IgG3 responders [28,37], some heterozygote phenotypes may also lead to low IgG3 responses.

It has been reported that *KM*3/KM*3* homozygote CF patients had higher levels of IgG2 and IgG3 antibodies to *Ps. aeruginosa* sonicate [20], and this genotype has also been associated with a higher IgG2 response to polysaccharides compared with *KM*3/KM*1* heterozygotes [39]. Interestingly, we found higher IgG1 aβab levels in *KM*3/KM*3* homozygotes compared with *KM*3/KM*1* heterozygotes.

In conclusion, our results show, in agreement with previous studies, that G2M and G3M genetic markers determine IgG2 and IgG3 aβab levels, and that IgG3 aβab are associated with poor lung function and IgG4 aβab with good lung function in patients with CF, as also found for other *Ps. aeruginosa* antigens. Our data suggest a possible relationship between the clinical course of pulmonary disease in patients with CF and their GM allotypes.

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